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Antimicrobial Susceptibilities of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* spp. Isolated in Spain

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The susceptibilities of 143 *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* isolates to 18 antimicrobial agents were tested. All *P. gingivalis* isolates were susceptible. In contrast, some *Prevotella* spp. (17%) were resistant to β-lactams, erythromycin, clindamycin, or tetracycline and carried resistance genes, *ermF* or *tetQ*, or β-lactamases.

Black-pigmented, gram-negative oral anaerobes such as *Porphyromonas gingivalis* and the *Prevotella intermedia-Prevotella nigrescens* group are thought to be pathogens in adult periodontitis (11). In addition, the *P. intermedia-P. nigrescens* group may be involved in both oral and nonoral infections (7, 8). Patients who do not respond to common surgical or mechanical periodontal therapy are often administered antibiotics as a complement to conventional treatment (12). However, limited susceptibility data has been available for these bacteria and no separate breakpoints have been established by the National Committee for Clinical Laboratory Standards (NCCLS) (9).

The purpose of this study was to examine the susceptibilities of oral isolates of *P. gingivalis* and *P. intermedia-P. nigrescens* to 18 antimicrobial agents and to determine if there is a correlation between the MIC and the presence of known antibiotic resistance genes.

A total of 143 clinical isolates were obtained from different patients diagnosed with adult periodontitis at the School of Stomatology of the University of Oviedo, Oviedo, Spain, between 1995 and 1997. None of the patients had been given antibiotic therapy within the previous 6 months. The isolates were the most prevalent anaerobic species from oral samples collected from the supragingival plaque by means of sterile cotton pledgets and subgingival samples collected by using two sterile paper points, which were inserted at each study site. The API Rapid ID32 A system (bioMérieux, Marcy-l'Étoile, France) was used for initial identification. The *P. intermedia-P. nigrescens* isolates were verified with a 16S rRNA-based PCR assay, as described by Conrads et al. (4). *P. gingivalis* was also verified by PCR, as previously described (1).

The antibiotics used for susceptibility testing were as follows: benzylpenicillin and tetracycline (Antibióticos, S.A., Madrid, Spain); amoxicillin, ampicillin, and ticarcillin (Smith Kline & French, S.A., Madrid, Spain); piperacillin (Lederle Laboratories, Pearl River, N.Y.); cephalothin (Lilly, S.A., Madrid, Spain); cefuroxime (Glaxo Group Research Ltd., Greenford, United Kingdom); cefotaxime (Hoechst Farma, S.A., Madrid, Spain); cefoxitin and imipenem (Merck Sharp & Dohme de España, Madrid, Spain); erythromycin (C.E.P.A., Madrid, Spain); cefamandole, cephaloridine, and oleandomycin (Sigma Chemical Co., St. Louis, Mo.); metronidazole and spiramycin (Rhône-Poulenc Rorer, S.A., Madrid, Spain); and clindamycin (Upjohn Farmoquímica, Madrid, Spain). MICs were determined under anaerobic conditions at 37°C by the agar dilution method described by the NCCLS using Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% sterile defibrinated sheep blood (9). Serial dilutions of the antibiotics ranging from 128 to 0.125 μg/ml were prepared and used on the same day. The final inoculum contained approximately 10^5 CFU per spot. Quality control strains (*Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741) were included with each run. Plates were read at 48 h. The MIC was defined as the lowest antimicrobial concentration which prevented visible growth of bacteria.

All 31 *P. gingivalis* isolates were susceptible to all of the antibiotics tested (Table 1), which is similar to previous findings (10). In contrast, some of the *P. intermedia* and *P. nigrescens* isolates were resistant to various antibiotics. We found that 14% (14 *P. intermedia* and 2 *P. nigrescens* isolates) were resistant to β-lactam antibiotics (penicillins and/or some cephalosporins). Cefinase disks (BBL Microbiology Systems, Cockeysville, Md.) were used to examine β-lactamase production. All 16 isolates had β-lactamase activities and had high MICs to penicillins and/or cephalosporins (Table 1). The percentage of β-lactamase-positive isolates observed in the *P. intermedia-P. nigrescens* group (14%) was lower than the 26% that has been reported previously (2, 14). The MICs for the cephalosporin-resistant strains of this study were similar to those exhibited for oral isolates of *P. intermedia* encoding a 2e cephalosporinase that we characterized recently (13).

Ninety percent of *P. intermedia* isolates were susceptible to erythromycin (MIC_{90}, 1 μg/ml), oleandomycin (MIC_{90}, 1 μg/ml), spiramycin (MIC_{90}, 0.125 μg/ml), and clindamycin (MIC_{90}, ≤0.125 μg/ml). All isolates of the *P. intermedia-P. nigrescens* group were susceptible to spiramycin (MIC_{90}, 0.125 μg/ml). Although no macrolide breakpoints are currently available for these anaerobes, we found high erythromycin, oleandomycin, and clindamycin MICs for two strains of *P. nigrescens* (15.4%). PCR assays for the *ermF* gene were performed with primers F_1 (5′ CGGGTCAGCACTTTACTATTG 3′) and F_2 (GGACCTACCTCATAGACAAG 3′), and the PCR products

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Susceptibilities of *P. gingivalis*, *P. intermedia*, and *P. nigrescens* to antimicrobial agents

Antimicrobial agent	MIC breakpoint (μg/ml)[a]	MIC (μg/ml)								
		P. gingivalis (31)[b]			*P. intermedia* (99)			*P. nigrescens* (13)		
		Range	50%[c]	90%	Range	50%	90%	Range	50%	90%
Penicillin	≤0.5	≤0.125–0.5	≤0.125	0.25	≤0.125–16	≤0.125	1	≤0.125–16	≤0.125	1
Ampicillin	≤0.5	≤0.125–2	≤0.125	0.25	≤0.125–8	0.25	0.5	0.25–8	0.25	0.25
Amoxicillin	NA	≤0.125–1	≤0.125	0.25	≤0.125–8	0.25	1	≤0.125–8	0.25	0.25
Ticarcillin	≤32	≤0.125	≤0.125	≤0.125	≤0.125–16	≤0.125	2	≤0.125–16	≤0.125	0.25
Cephaloridine	NA	≤0.125–2	≤0.125	2	0.25–32	≤0.125	32	≤0.125–32	≤0.125	0.25
Cephalothin	NA	≤0.125–2	≤0.125	2	0.25–≥128	0.25	≥128	≤0.125–≥128	0.25	0.25
Cefamandole	≤8	0.25–2	0.25	0.5	0.25–4	0.25	4	≤0.125–4	0.25	1
Cefotaxime	≤16	0.25	0.25	0.25	≤0.125–4	0.5	4	≤0.125–4	0.25	2
Cefoxitin	≤16	0.25	≤0.125	≤0.125	≤0.125–2	0.5	2	≤0.125–2	0.5	2
Cefuroxime	NA	≤0.125–0.5	≤0.125	0.25	≤0.125–64	0.25	4	≤0.125–64	0.25	4
Imipenem	≤4	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125
Piperacillin	≤32	≤0.125–2	0.25	1	≤0.125–16	0.25	1	≤0.125–16	≤0.125	1
Erythromycin	NA	≤0.125–1	0.25	1	0.25–0.5	0.5	1	≤0.125–8	0.5	1
Oleandomycin	NA	≤0.125–2	0.5	1	0.5–1	0.5	1	≤0.125–16	0.5	1
Spiramycin	NA	0.125	0.125	0.125	0.125–0.25	0.125	0.125	0.125–0.25	0.125	0.125
Clindamycin	≤2	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125–≥256	≤0.125	≤0.125
Tetracycline	≤4	≤0.125–0.5	0.25	1	≤0.125–1	0.25	1	≤0.125–16	≤0.125	16
Metronidazole	≤8	0.125–2	0.125	0.125	0.125–32	0.125	0.125	0.125–1	0.125	0.125
Metronidazole-spiramycin	NA	≤0.032–0.125	≤0.032	≤0.032	≤0.032–0.125	≤0.032	≤0.032	≤0.032–0.125	≤0.032	≤0.032

[a] Breakpoints correspond to susceptibility values recommended by the NCCLS (9). NA, not available.
[b] Numbers in parentheses indicate the numbers of strains tested.
[c] 50% and 90%, MICs required to inhibit 50 and 90% of the isolates, respectively.

were confirmed by hybridization. Both resistant isolates were positive for the *ermF* gene.

Tetracycline inhibited 90% of the *P. intermedia* isolates (MIC$_{90}$, 1 μg/ml) and was less effective against the isolates of *P. nigrescens* (MIC$_{90}$, 16 μg/ml). By a previously described PCR assay for *tetQ* (7), two tetracycline-resistant *P. nigrescens* isolates were shown to carry the *tetQ* gene; one isolate also carried the *ermF* gene. The incidence of tetracycline resistance observed in this study for the *P. intermedia-P. nigrescens* group (3.6%) was much lower than the 26% reported previously for *Prevotella* spp. from other areas (6).

Metronidazole was highly active with the *P. intermedia-P. nigrescens* group (MIC$_{90}$, 0.125 μg/ml). Resistance (32 μg/ml) was found in three isolates of *P. intermedia*. All isolates were susceptible to metronidazole-spiramycin (1:1.48), which is a combination used commonly in Spain for treatment of a variety of dental infections. The MIC$_{90}$ of this combination (≤0.032 μg/ml) was much lower than the MIC$_{90}$ of each antimicrobial agent tested separately, suggesting a synergistic effect that has been reported previously (3).

We have been able to link three classes (β-lactam, macrolide-lincosamide, and tetracycline) of antibiotic resistance with known mechanisms of resistance (β-lactamases, rRNA methylases encoded by the *ermF* gene, or a ribosomal protection protein encoded by the *tetQ* gene) in the *P. intermedia-P. nigrescens* group. All three types of genes have been associated with conjugative elements in oral *Prevotella* spp. (5, 15), and gene transfer between different species has been demonstrated in the laboratory (5, 15). Thus, the *P. intermedia-P. nigrescens* group may function as an antibiotic resistance gene reservoir and may influence the success of antibiotic therapy in the oral cavity.

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